Characterisation of a mural cell network in the murine pituitary gland

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Abstract

The anterior and intermediate lobes of the pituitary are composed of endocrine cells, vasculature and supporting cells, such as folliculostellate cells. Folliculostellate cells form a network with several postulated roles in the pituitary, including production of paracrine signalling molecules and cytokines, coordination of endocrine cell hormone release, phagocytosis and structural support. Folliculostellate cells in rats are characterised by expression of S100B protein, and in humans by GFAP. However, there is evidence for another network of supporting cells in the anterior pituitary that has properties of mural cells such as vascular smooth muscle cells and pericytes.

This study aims to characterise the distribution of cells that express the mural cell marker PDGFRβ in the mouse pituitary and establish whether these cells are folliculostellate. By immunohistochemical localisation we determine that approximately 80% of PDGFRβ+ cells in the mouse pituitary have a non-perivascular location, and 20% are pericytes. Investigation of gene expression in a magnetic cell sorted population of PDGFRβ+ cells shows that, despite a mostly non-perivascular location, this population is enriched for mural cell markers, but not enriched for rat or human folliculostellate cell markers. This is confirmed by immunohistochemistry.

This study concludes that a mural cell network is present throughout the anterior pituitary of the mouse, and that this population does not express well-characterised human or rat folliculostellate cell markers.

Key words: Pituitary, folliculostellate, folliculo-stellate, mural, SOX2

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Introduction

The pituitary is an endocrine gland located underneath the brain that produces and releases circulating hormones in response to signals from the hypothalamus. It is formed of three lobes. Neurons in the posterior lobe (pars nervosa) connect to the hypothalamus and store oxytocin and vasopressin. The intermediate lobe (pars intermedia) is formed of melanotrophs that produce melanocyte stimulating hormone (MSH), and the anterior lobe (pars distalis) is formed of heterogeneous specialised endocrine cells that each make a particular hormone type: adrenocorticotrophic hormone (ACTH) is made by corticotrophs, growth hormone (GH) by somatotrophs, prolactin by lactotrophs, thyroid stimulating hormone (TSH) by thyrotrophs and the gonadotrophins follicle stimulating hormone (FSH) and luteinising hormone (LH) by gonadotrophs. The folliculostellate cell is a further, non-endocrine cell type in the anterior pituitary. Folliculostellate cells are named after their appearance in electron micrographs: they have a distinctive stellate shape and surround follicular structures [1]. Their long cytoplasmic processes connect to form a three-dimensional network [2] that is thought to perform multiple roles in the pituitary including production of paracrine signalling molecules and cytokines [1], coordination of endocrine cell hormone release [3], phagocytosis [4] and structural support [5]. There is evidence that folliculostellate cells in rats are composed of multiple subpopulations that may arise from different precursors [6].

Mural cells such as vascular smooth muscle cells and pericytes are required for the formation and stability of the vasculature and the support of endothelial cells. They are characterised by the expression of the markers Chondroitin Sulfate Proteoglycan 4 (CSPG4, more commonly known as nerve/glial antigen 2 or NG2), alpha smooth muscle actin (SMA) and platelet derived growth factor receptor beta (PDGFRβ) [7, 8]. In rat anterior pituitaries they have also been shown to express desmin [9]. A recently published paper has shown that mouse folliculostellate cells express NG2, and that inactivation of retinoblastoma protein in NG2+ cells resulted in adenohypophysial tumours with immunohistochemical and ultrastructural features that resemble those of aggressive Pit1-lineage tumours in humans [10]. Furthermore, PDGFRβ has been shown to be expressed in the mouse TtT/GF folliculostellate cell line [11]. Together, this evidence suggests that either mouse pituitary folliculostellate cells may have mural cell-like properties, or mural cells form a network similar to that of folliculostellate cells in the anterior pituitary. This study aims to

characterise the distribution of PDGFRβ+ cells in the mouse pituitary and establish whether these cells have folliculostellate cells and/or mural-like properties.

Methods

Breeding and maintenance of mice

Mice were fed a soya-free diet. Experimental procedures and animal breeding and maintenance were approved by University of Edinburgh Animal Welfare and Ethical Review Body and were carried out with licenced permission under project licence 70/8804 held by Professor Lee B. Smith in line with the UK Home Office Animals (Scientific Procedures) Act, 1986.

Immunohistochemistry

NG2 staining was only possible with cryopreserved sections. To co-stain for NG2 and PDGFRβ, Pituitaries were frozen on dry ice and transferred to a -80°C freezer. Prior to sectioning, pituitaries were encompassed in Tissue-Tek OCT compound (Sakura), mounted on a cryostat chuck, sectioned at 5 µm and mounted on poly lysine coated slides (Sigma-Aldrich PO425-72EA). Sections were dried at room temperature for 12 hours, then sections fixed under a droplet of 4% buffered formaldehyde for 5 minutes. Slides were then stained with a double fluorescent tyramide method as described previously [15, 16] using antibodies to NG2 (Millipore Cat# AB5320, RRID:AB_91789, 1 in 8000 dilution) and PDGFRβ (Abcam Cat# ab32570, RRID:AB_777165, 1 in 8000 dilution). A non-pressurised heating by microwave in pH6 0.01M sodium citrate buffer was performed after the first tyramide reaction to denature remaining antibodies from the first set and avoid non-specific reactions with the second set of antibodies[17]. Double staining of either NG2 first or PDGFRβ first, both with and without primary antibodies, was performed to verify this (Supplementary Data 4).

All other antibodies could be used on formaldehyde-fixed paraffin-embedded sections. Pituitaries were fixed for 24 hours in 4% buffered formaldehyde. Fixed tissues were processed and embedded in paraffin wax, and 3µm sections were used for immunohistochemical analysis.

Fluorescent immunostaining was performed either by single or double antibody tyramide fluorescent immunostaining method, as described previously [15, 16]. Antibodies used were PDGFRβ (Abcam Cat# ab32570, RRID:AB_777165, 1 in 1000), S100 (Agilent Cat# Z0311, lot number 20038616, RRID:AB_10013383, 1 in 2000) and SOX2 (Abcam Cat# ab92494, RRID:AB_10585428 1

in 500) optimised for the method and conditions used. A non-pressurised heating by microwave in pH6 0.01M sodium citrate buffer was performed after the first tyramide reaction to denature remaining antibodies from the first set and avoid non-specific reactions with the second set of antibodies [17].

On resin-perfused sections, staining was performed using a colourimetric method. Briefly, sections were deparaffinised and rehydrated, and high-pressure antigen retrieval was performed in citrate buffer. Slides were incubated with NGS/TBS/BSA to block non-specific antibody binding, before being incubated overnight with rabbit anti- PDGFRβ (Abcam Cat# ab32570, RRID:AB_777165, 1 in 400) in NGS/TBS/BSA. The next day, slides were incubated with biotinylated goat anti-rabbit (Vector Laboratories Cat# BA-1000, RRID:AB_2313606, 1 in 500), then streptavidin-conjugated alkaline phosphatase (Vector Laboratories Cat# SA-5100, RRID:AB_2336093, 1 in 200), then PermaBlue Plus/AP (Diagnostic BioSystems K058, diluted to manufacturer's instructions) to form a blue precipitate localised to PDGFRβ protein. Slides were then mounted using an aqueous mounting medium and visualised with a Provis light microscope and camera.

Magnetic activated cell sorting (MACS)

Pituitaries were removed during dissection and the anterior lobes were dissected and used for MACS. Anterior pituitaries from approximately 40 mice on a mixed C57Bl/6 background were used in each MACS experiment, and MACS was repeated three times. The pool of anterior pituitaries was digested into a single cell suspension following a previously published protocol [18]. MACS was performed using protocols and reagents from Miltenyi Biotec (Bergisch Gladbach, Germany). Briefly: the single cell suspension was incubated first with 1:500 PDGFRβ antibody (Abcam Cat# ab32570, RRID:AB_777165), then with anti-rabbit IgG magnetic microbeads (Miltenyi 130-048-602) before passing through a Miltenyi MS column (Miltenyi 130-042-201) in a magnetic holder. Both the column-bound cell fraction (MACS+) and the flow-through cell fraction were kept, washed and pelleted.

Preparation of cDNA

Recovered whole pituitaries, dissected pituitaries and MACS cell pellets were frozen on dry ice before being transferred to a -80°C freezer for storage. RNA was isolated using the RNeasy Mini extraction kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. RNA was quantified and assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All RNA samples had a A260/A280 ratio of above 1.9, and a A260/A230 ratio of above 1.5. Random hexamer primed cDNA was prepared using the SuperScript VILO cDNA synthesis kit (Life Technologies) according to manufacturer's instructions.

Quantitative RT-PCR (qPCR)

Multiplex qPCR was performed on cDNA for the genes listed in Table 1 using an ABI Prism 7900 Sequence Detection System (Applied Biosystems) and the Roche Universal Probe library (Roche, Welwyn, UK). The expression of all genes was related to an internal housekeeping gene assay for *Actb* (Roche, Welwyn, UK) as described previously [16]. Actb Ct values were similar between samples when normalised to the number of cells that had been RNA extracted. Resulting data were analysed using the ΔΔCt method.

Resin perfusion

Resin perfused pituitaries were obtained from Dr Diane Rebourcet (University of Newcastle, Australia), prepared following previously published protocols [19].

Immunogold Electron microscopy

Pituitaries were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer, pH 7.2 at room temperature for 3 hours, then transferred to 0.25% glutaraldehyde and 0.2% paraformaldehyde in 0.1M phosphate buffer, pH 7.2 and then kept at 4°C. Pituitaries were prepared for immunogold electron microscopy as previously described [20]. Briefly, cells were post-fixed in osmium tetroxide (1% wt/vol in 0.1 M sodium phosphate buffer), contrasted with uranyl acetate (2% wt/vol in distilled water), dehydrated through increasing concentrations of ethanol (70–100%) and embedded in Spurr's resin. Ultra-thin sections (50–80 nm) cut and stained with antibody to PDGFRβ (Abcam Cat# ab32570, RRID:AB_777165). Sections were viewed with a JEM-1010 transmission electron microscope (JEOL USA Inc., Peabody, MA, USA).

Results

PDGFRβ cells were found in the anterior, intermediate and posterior lobes of the mouse pituitary

Stellate PDGFRβ cells were widely distributed in the anterior and posterior lobes of the adult pituitary, with fewer seen in the intermediate lobe (Figure 1A). PDGFRβ cells were also present in the day (d)1 neonatal anterior pituitary as stellate shaped cells and cell clusters and in a layer of spindle-shaped cells around the outside of the anterior pituitary (Figure 1B). Mural pericytes have a characteristic location and morphology, defined as being adjacent to endothelial cells and wrapping around the vasculature [21]. In the rat anterior pituitary, desmin-positive perivascular cells can be found detached from capillaries, although with cytoplasmic processes still in contact with the capillary wall [22]. To visualise whether PDGFRβ+ cells are found only in a perivascular location in the anterior pituitary, we immunostained for PDGFRβ in sections of pituitary from adult mice that had been intravascularly perfused with an opaque resin at termination [19]. Both large and small blood vessels can be visualised by this technique. The percentage of PDGFRβ+ cells in contact with and not in contact with blood vessels was then quantified. Although 20% of PDGFRβ+ cells were seen to be either contacting or surrounding anterior pituitary blood vessels in a location characteristic of pericytes, 80% were located in the pituitary parenchyma in a location and distribution characteristic of folliculostellate cells (Figure 1C). To identify PDGFRβ cells by ultrastructure, immunogold electron microscopy was performed with a PDGFRβ antibody. Folliculostellate cells and pericytes were identified by location and ultrastructure (Supplementary data 3). Both folliculostellate cells (Figure 2A) and pericytes (Figure 2B) were found to localise PDGFRβ.

PDGFRβ cells of the anterior pituitary were found to express other mural cell markers

To investigate whether pituitary PDGFRβ+ cells express other mural cell markers, a population of cells enriched for PDGFRβ was generated by MACS from micro-dissected anterior pituitaries using the same PDGFRβ antibody used for immunohistochemistry (Figure 3A). These markers were examined by qPCR in whole pituitaries and dissected anterior pituitaries, as well as PDGFRβ positive and negative purified MACS populations.

Cspg4 (NG2 transcript) was enriched in a PDGFRβ MACS+ population at 10-fold higher than the MACS- population, 7-fold higher than the whole anterior pituitary and 7-fold higher than the whole intact pituitary (Figure 3B). This was confirmed by immunohistochemistry for NG2 protein in cryosections of whole mouse pituitary. Staining of NG2 colocalised with PDGFRβ in the anterior lobe in a stellate shaped cell pattern (Figure 3C). Other mural cell markers were enriched in the PDGFRβ cell population of the mouse pituitary. *Acta2* (SMA transcript) was enriched in the MACS+ population at 5-fold higher than the MACS- population, 9-fold higher than the whole anterior pituitary and 2-fold higher than the whole intact pituitary PDGFRα transcript (Figure 3D). Desmin transcript *Des* was enriched in the MACS+ population at 11-fold higher than the MACS- population, 3-fold higher than the whole anterior pituitary and 3.5-fold higher than the whole intact pituitary (Figure 3E). Since PDGFRβ is also considered a marker of mesenchymal stem cells (MSCs)[23], PDGFRβ enriched cell population was also tested for *Pdgfra*, a MSC marker. *Pdgfra* [24] was enriched in the MACS PDGFRβ + population at 12.5-fold higher than the MACS- population, 2.5-fold higher than the whole anterior pituitary and 1.4-fold higher than the whole intact pituitary (Figure 3F). These results indicate that the PDGFRβ+ cell population in the anterior pituitary is enriched for mural cell and perivascular MSC markers.

PDGFRβ cells of the mouse anterior pituitary did not express rat or human folliculostellate cell markers

We then investigated whether pituitary PDGFRβ cells express the same markers as rat and human folliculostellate cells. These markers were examined by qPCR in whole pituitaries and dissected anterior pituitaries, as well as positive and negative purified MACS populations. Sections of whole pituitary were also stained for the markers where an appropriate antibody was available.

Localisation of S100 calcium-binding protein B (S100B) is a characteristic of folliculostellate cells in rats [25]. *S100b* transcript was not enriched in the MACS+ population compared to the MACS- population and was present at 1.6-fold lower than the whole anterior pituitary and 4-fold lower than the level of the whole intact pituitary. (Figure 4A). Since it was found to be enriched in whole pituitaries compared to dissected anterior pituitaries, it is likely that it is enriched in intermediate or posterior lobe cells. This was confirmed by immunohistochemistry for pan-S100

protein in whole mouse pituitary sections. S100 staining was not seen in the parenchyma of the anterior lobe (Figure 4B) but was seen in a population of cells lining the cleft on both the AP and IP sides (Figure 4C). As positive controls to ensure that the antibody is functional, staining could be seen both in astrocyte and tanycyte cells in the mouse hypothalamus (Figure 4D) and in sections of rat pituitary (Figure 4E), as expected. Glial fibrillary acid protein (GFAP) is a folliculostellate cell marker in humans [26]. *Gfap* was not enriched in the MACS+ population compared to the MACS- population or compared to whole anterior pituitary but was present in whole pituitaries at 14.5-fold the level of the MACS+ cell population (Figure 4F). Since it was highly enriched in whole pituitaries compared to dissected anterior pituitary samples, it is likely that it is enriched in intermediate or posterior lobe cells. This was confirmed by immunohistochemistry for GFAP protein in whole mouse pituitary sections. Staining was not seen in the parenchyma of the anterior lobe (Figure 4G). As a positive control to ensure that the antibody is functional, staining could be seen in astrocytes in the mouse brain (Figure 4H). These results indicate that the PDGFRβ+ cell population in the anterior pituitary is not enriched for rat or human folliculostellate cell markers.

SOX2+ cells in the adult pituitary gland did not express PDGFRβ

To clarify whether PDGFRβ+ cells in the pituitary express the previously characterised pituitary stem cell marker SOX2 we performed double immunohistochemistry for these proteins. PDGFRβ+ cells did not co-stain with SOX2 in adult pituitaries (Figure 5A) or in neonatal pituitaries (Supplementary Data 1).

Discussion

A network of mural cells in the mouse pituitary has a spatial distribution similar to folliculostellate cells

In this study we have shown that staining for PDGFRβ is present throughout the mouse pituitary in a network of cells with a stellate shape. PDGFRβ is a marker of mural cells such as pericytes and vascular smooth muscle cells, and also of mesenchymal stem cells (MSCs) [23] and fibroblasts [27] of some tissues. Although 20% of the cells seen to stain for PDGFRβ in the anterior pituitary interact with blood vessels, 80% did not make contact. The expression pattern of PDGFRβ in the pituitary parenchyma was similar to previous staining of folliculostellate cells in the mouse pituitary using an antibody to ER-BMDM1 [28].. Since other cells such as MSCs and fibroblasts express PDGFRβ, we aimed to characterise whether mouse anterior pituitary parenchymal PDGFRβ+ cells expressed any other mural cell markers. In this study we have shown that anterior pituitary PDGFRβ+ cells co-stain with NG2 which is also a marker of mural cells, but not MSCs [23] or fibroblasts [29]. This expression pattern is consistent with a previous publication which has also localised NG2 to a network of cells with a stellate shape, although that paper designated the cells as mouse pituitary folliculostellate cells [10]. A few cells appear to stain for NG2 or PDGFRβ only. This could be an artefact of the staining, or it could be that there are some cells in the anterior pituitary that are NG2+/PDGFRβ- and NG2-/ PDGFRβ+. Without further investigation it is difficult to conclude which possibility is true. Our evidence suggests that a population of mural cells in the mouse anterior pituitary has a network structure very similar to that of folliculostellate cells, and that this network extends away from the vasculature.

The novel mural cell network does not express folliculostellate cell markers

The folliculostellate cells of the pituitary also form a network, which is commonly characterised by expression of S100B or GFAP markers in immunohistochemistry. 473 papers published between 1972 and November 2019 that mention the words “folliculostellate” or “folliculo-stellate” in their abstract have been indexed on Pubmed. Of these, 426 are primary research papers and 47 are reviews. 202 of the 426 primary research papers have used rats as their animal

model to investigate pituitary folliculostellate cells, either in whole pituitaries, primary cell culture or derived cell lines. Folliculostellate cells in rats are defined by their expression of the calcium binding protein S100B but are considered functionally heterogeneous, arising from three different developmental origins [6]. Astrocyte-like folliculostellate cells express glial fibrillary acidic protein (GFAP) as a marker, dendritic cell-like folliculostellate cells are thought to arise from MAC-1+ early macrophages and express MH-class II as a marker, and epithelial-like folliculostellate cells express neither of these. The use of S100B as a marker and the availability of a S100-GFP reporter rats [25] has resulted in the rat becoming the most widely used model organism for folliculostellate cell research, and most folliculostellate cell characterisation is based on their phenotype in rats. Pericytes and folliculostellate cells are distinct populations in rats [30], but there is also evidence in rats that pericyte-like cells may be found in contact with (but not enveloping) capillaries [22].

Mouse folliculostellate cells are not well-investigated compared to rats and only 25 of these 423 primary research papers use mice as a model. To determine whether the network of PDGFRβ cells we characterised in the mouse anterior pituitary expressed the rat folliculostellate cell marker S100B or the human folliculostellate cell marker GFAP, we attempted to detect these proteins by immunohistochemistry on whole anterior pituitaries and transcripts by qPCR on a MACS purified population of PDGFRβ cells. Our results showed that GFAP is not expressed in the anterior pituitary, and S100 is only expressed in cells of the anterior pituitary lining the pituitary cleft, and not in the body of the anterior pituitary. Neither GFAP or S100 are enriched in mouse anterior pituitary PDGFRβ cells. However, utilising immunogold electron microscopy for PDGFRβ shows it localised to cells with ultrastructural characteristics of both folliculostellate cells and pericytes. Previous studies (including those that have used the same antibody as we used in this study) have shown S100 staining in the pituitary [31-37], although these S100-positive cleft cells are often defined as 'folliculostellate cells' but do not show staining throughout the parenchyma. Interestingly, Allaerts et al. [38] note that they do not see S100 staining in the mouse pituitary. The S100+ cells found in the cleft are also likely to be SOX2+ due to their location and previous reports of co-staining of SOX2 and S100 in the rat [39].

A further 54 papers have been published using the TtT/GF cell line, which is an immortal cell line generated from a mouse thyrotropic pituitary tumour [40], classified as 'folliculostellate-like' based

on strong immuno-positive staining for GFAP and weak staining for S100 protein [40]. The TtT/GF cell line was isolated from a mouse thyrotrophic tumour that had been induced by radiothyroidectomy so it is possible that the transformation of these cells and their culture *ex vivo* has changed the characteristics of the cell line from that of the originating cell *in vivo*. However, it is also interesting to note that a recent paper discusses that the TtT/GF cell line has 'pericyte characteristics' including the expression of NG2 [41]. It is likely that this is a folliculostellate cell line but has either switched on or increased expression of S100 as well as mural cell markers, or that it has been derived from a sub-population of folliculostellate cells [6], potentially the from the SOX2+ S100+ population of cleft cells. Our evidence suggests that the mural cell network in the mouse anterior pituitary are not folliculostellate cells, although we could not establish a folliculostellate marker to use to unambigulously determine this, so there is still the possibility that these two networks overlap.

SOX2+ cells of the anterior pituitary are not PDGFRβ+

The source of dividing cells in the anterior pituitary is thought to change throughout development. Previous studies show that the very earliest progenitor cells in the developing pituitary come from SOX2+ cells lining Rathke's pouch, which at e12.5 ubiquitously express the transcription factor SOX2 and are highly proliferative [43]. From e12.5 to e17.5, between 20-30% of SOX2+ cells are dividing at any given point [44]. By E14.5, SOX2 is confined to the intermediate lobe and periluminal cells in the anterior lobe and by e16.5, dividing cells begin to switch off SOX2 and switch on the lineage-specific transcription factors TPIT (a.k.a. TBX19: corticotrophs and melanotrophs), SF1 (gonadotrophs) and PIT1 (a.k.a. POU1F1: thyrotrophs, somatotrophs and lactotrophs) [33]. Lineage tracing studies that use a tamoxifen-inducible *Sox2*-Cre to permanently express fluorescent marker proteins in these cells and their progeny have shown that all types of pituitary anterior lobe endocrine cells can develop from early SOX2+ cells, but not all cells are marked by the lineage tracer [31, 32, 43]. A small number of SOX2+ cells persist in the postnatal pituitary, found lining the anterior-pituitary slide of the cleft and in small groups through the parenchyma of the anterior pituitary. When isolated and cultured, these cells form pituispheres

which can differentiate into all types of pituitary endocrine cells, which has led to the suggestion that they might be pituitary stem cells [33].

In the first three weeks of postnatal life, the pituitary undergrows rapid growth in which differentiated or differentiating endocrine cells increase their contribution to cell division, and SOX2+ cells decrease their contribution [45]. SOX2+ cells contribute around 64% of dividing cells at d1, reduced to 19% at d10 and are mostly quiescent at d20. In contrast, the percentage of dividing cells that are Pit1-positive (a marker of differentiating lactotrophs, somatotrophs and thyrotrophs) increases from 16% to 87% over this time period [43]. Two lineage tracing studies for SOX2+ cells have investigated the early postnatal period and concluded that only a small percentage of SOX2+ cells present in the neonatal pituitary develop into pituitary endocrine cells [32, 43] and that most of the SOX2+ dividing cells develop into gonadotrophs.

In adulthood it is thought that the adult pituitary gland is maintained by stochastic cellular self-replication rather than stem cell replenishment, although stressing the pituitary by ablating a cell population or removing a feedback gland can prompt the mobilisation of SOX2+ stem cells. Lineage tracing experiments show that when *SOX2*-Cre is induced in adulthood, labelled cells rarely differentiate into different pituitary lineages and mostly remain as SOX2+ stem cells [31, 32, 43]. Taken together this evidence shows that SOX2+ stem cells have a large commitment to differentiating cells in embryonic period but a diminishing contribution after birth and little during adulthood. Our study shows that the SOX2+ cells lining the anterior epithelium of the pituitary cleft do not express PDGFRβ in adult or neonatal pituitaries.

Conclusion

The anterior pituitary is composed of well-characterised networks of endocrine and folliculostellate cells [52]. Here we present evidence that there is a population of mural cells in the anterior pituitary of the mouse that extends away from the vasculature to form a further network. The role of this mural cell network requires further characterisation: since pituitary vasculature is important for the coordination of hormone release [53], it is possible that it is involved in coordinating the communication between the vasculature and endocrine cells.

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Tables

Table 1. Primers and Roche UPL probes for qRT-PCR assays used in these studies

Gene	Forward primer	Reverse primer	Probe
Cspg4	cttggccttgttggtcagat	cacctccaggtggttctcc	16
Des	gccacctaccggaagctact	gcagagaaggtctggataggaa	15
Acta2	gacaccacccacccagagt	acatagctggagcagcgtct	20
Pdgfra	ggtcctcagctgtctcctca	cgtttgggaggatagagggta	51
Pdgfrb	gctgatgaaggtctcccaga	ggagctccaggggactgt	69
Gfap	acatcgagatcgccacctac	ggatctggaggttggagaaag	9

Figure legends

Figure 1: PDGFRβ cells are found in the anterior and posterior lobes of the pituitary

(A) Fluorescent immunohistochemical staining of PDGFRβ (magenta) in formaldehyde-fixed paraffin-embedded thin sections of wild-type mouse pituitary with green counterstain, showing anterior (left), intermediate and posterior lobes. (B) Anterior lobe of postnatal day (pnd) 1 mouse pituitary showing both clusters of stellate-shaped cells and cells around the periphery of the lobe staining for PDGFRβ. All scale bars 50 μm. (C) Perma-blue staining of PDGFRβ (blue) in thin sections of pituitary from mice intravascularly perfused with an opaque resin at termination, marking larger vessels in brown (arrow) and smaller vessels in iridescent green (arrowhead), showing that PDGFRβ-positive cells are present both adjacent to pituitary blood vessels and in the parenchyma, scale bar 50 μm.

Figure 2: PDGFRβ is localised to cells with folliculostellate and pericyte morphology

Immunogold electron microscopy was performed with a PDGFRβ antibody. (A) Localisation in folliculostellate cells identified from location and ultrastructure. (B) Localisation in pericytes identified from location and ultrastructure. Scale bars 200 nm.

Figure 3: **PDGFRβ cells of the anterior pituitary are enriched for mural cell markers**

(A) *Pdgfrb* is enriched in the MACS PDGFRβ+ population at 12.1-fold higher than the MACS- population, 2.5-fold higher than the whole anterior pituitary and 3.9-fold higher than the whole intact pituitary. (B) *Cspg4* (NG2 transcript) is enriched in a PDGFRβ MACS+ population at 10-fold higher than the MACS- population, 7-fold higher than the whole anterior pituitary and 7-fold higher than the whole intact pituitary. (C) Fluorescent immunochemistry for NG2 protein (magenta) in cryosections of whole wild-type mouse pituitary colocalises with PDGFRβ (green) in the anterior lobe in a stellate shaped cell pattern. Blue counterstain, scale bar 50 μm. (D) *Acta2* (SMA transcript) is enriched in the MACS+ population at 5-fold higher than the MACS- population, 9-fold higher than the whole anterior pituitary and 2-fold higher than the whole intact pituitary. (E) *Des* is enriched in the MACS+ population at 11-fold higher than the MACS- population, 3-fold higher than the whole anterior pituitary and 3.5-fold higher than the whole intact pituitary. (F) *Pdgfra* is enriched in the MACS PDGFRβ + population at 12.5-fold higher than

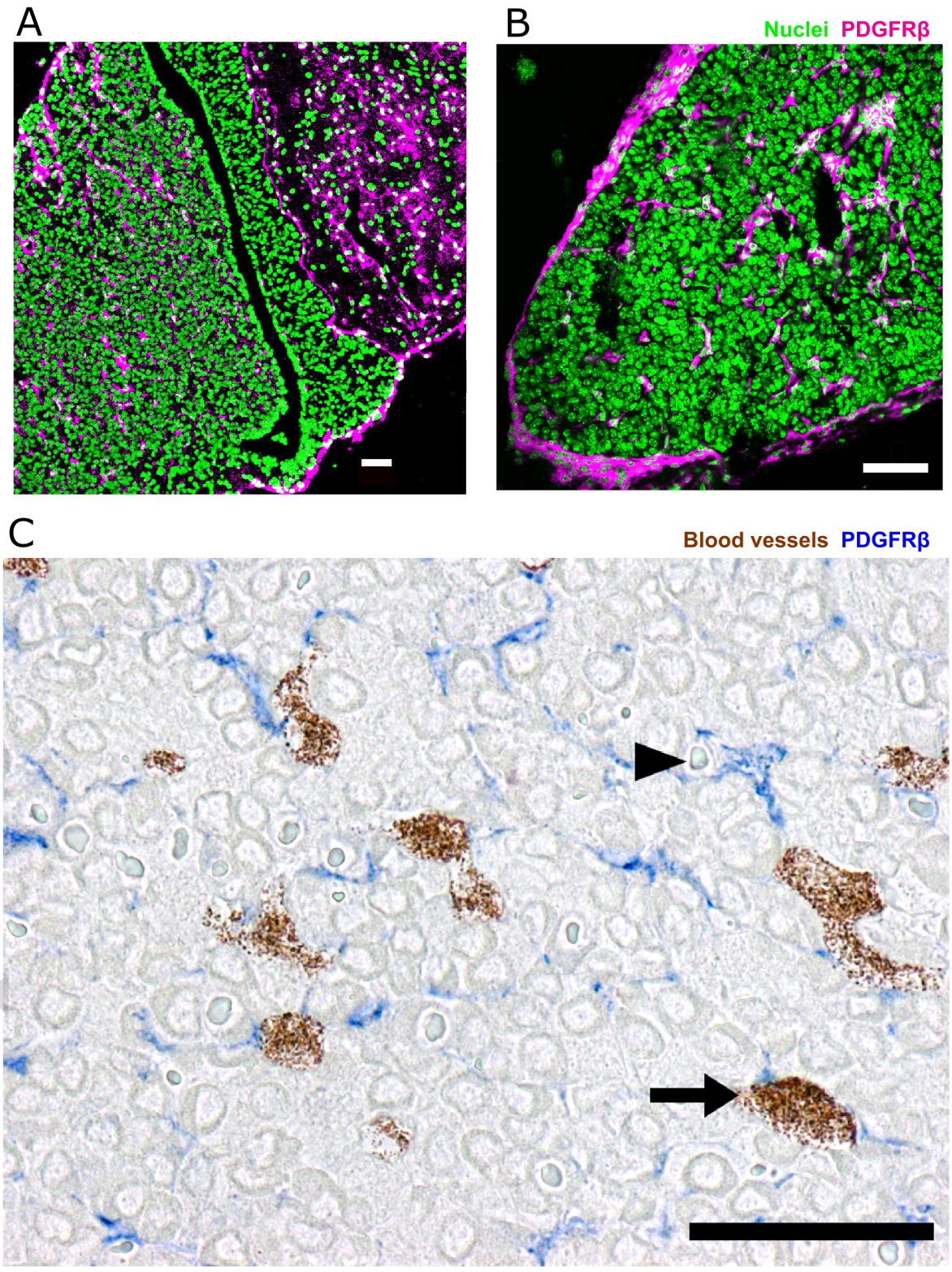
the MACS- population, 2.5-fold higher than the whole anterior pituitary and 1.4-fold higher than the whole intact pituitary.

Figure 4: PDGFRβ cells of the mouse anterior pituitary are not enriched for rat folliculostellate cell markers

(A) *S100b* is not enriched in the MACS+ population compared to the MACS- population and is present at 1.6-fold lower than the whole anterior pituitary and 4-fold lower than the level of the whole intact pituitary. (B-E) Fluorescent immunohistochemical staining of pan-S100 (magenta) in formaldehyde-fixed paraffin-embedded thin sections of wild-type mouse pituitary and brain with green counterstain, scale bars 50 μm: (B) Staining is not seen in the parenchyma of the anterior lobe of the pituitary. (C) Staining is seen in a population of cells lining the cleft on both anterior and intermediate sides. (D) As positive controls to ensure that the antibody is functional, S100 staining can be seen both in astrocyte and tanycyte cells in the mouse hypothalamus. (E) S100 staining is seen in folliculostellate cells in sections of rat pituitary. (F) *Gfap* is not enriched in the MACS+ population compared to the MACS- population, or compared to whole anterior pituitary, but is present in whole pituitaries at 14.5-fold the level of the MACS+ cell population. (G-H) Fluorescent immunohistochemical staining of GFAP (cyan) in formaldehyde-fixed paraffin-embedded thin sections with green counterstain, scale bars 50 μm: (G) GFAP staining is not seen in the parenchyma of the pituitary anterior lobe. (H) In mouse brain positive controls, GFAP staining can be seen in astrocytes in the mouse brain.

Figure 5: No colocalisation of SOX2 and PDGFRβ in anterior pituitary

(A) Fluorescent immunohistochemical staining of PDGFRβ (blue) and SOX2 (green) in formaldehyde-fixed paraffin-embedded thin sections of wild-type mouse pituitaries.



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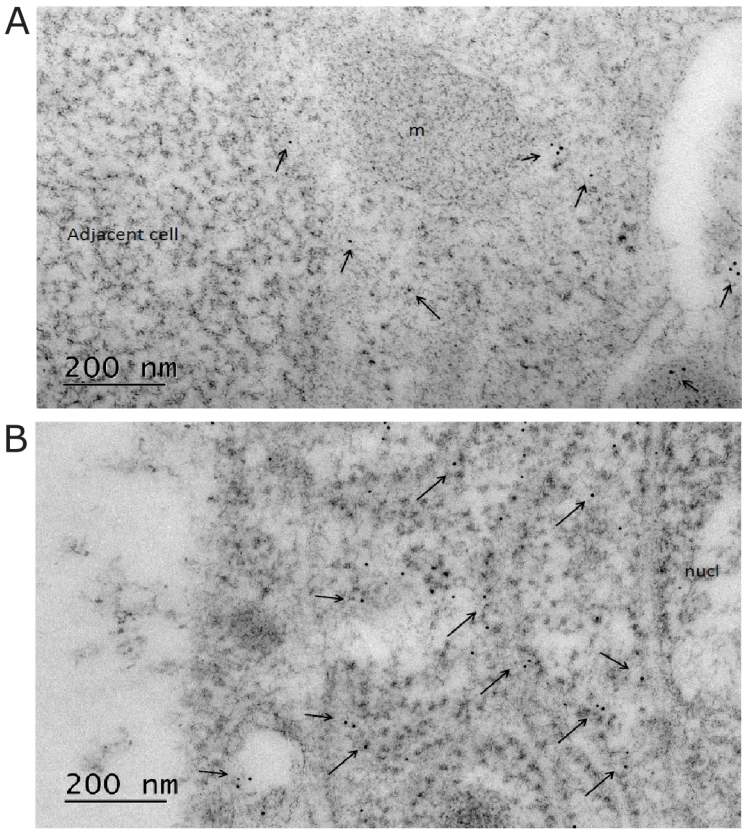

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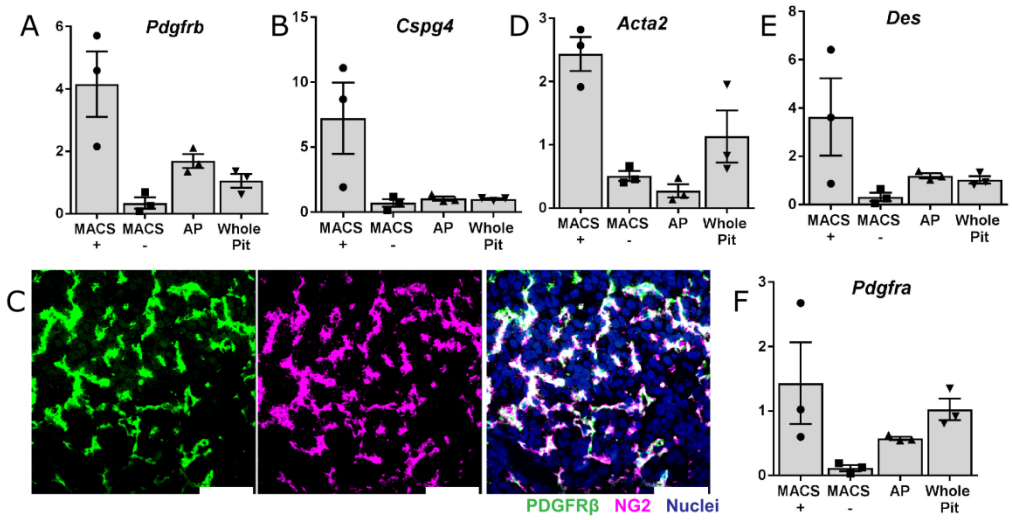

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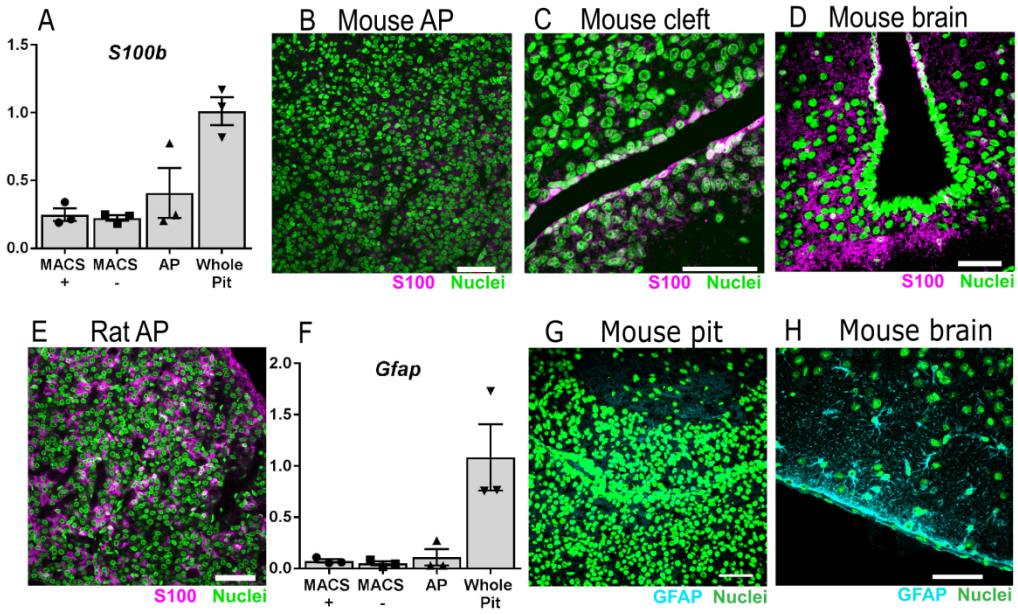

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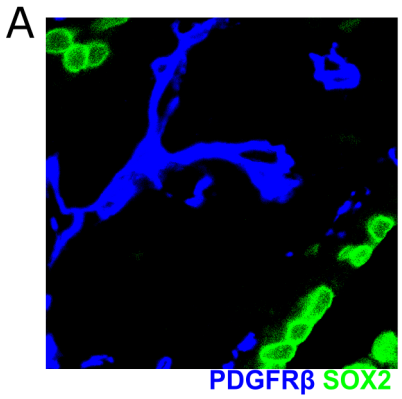

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